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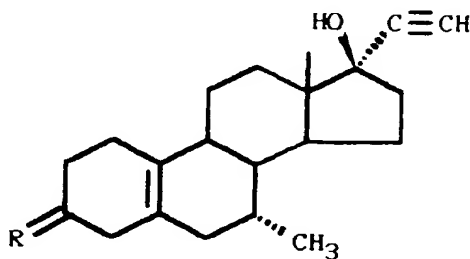
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NL PT SE**(71) Applicant: **Akzo Nobel N.V.**
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NL-5340 BH Oss (NL)(54) **Use of a pregnane derivatives for the treatment of tumours.**

(57) The invention relates to a use of pregnane derivatives of the following general formula:



in which R = H₂, (H₂O), (H₂OAcyl), or O, and especially the pregnane derivative (7 α ,17 α)-17-hydroxy-7-methyl-19-nor-17-pregn-5(10)-en-20-yn-3-one (compound I), for the manufacture of a medicament for the prevention or treatment of tumors.

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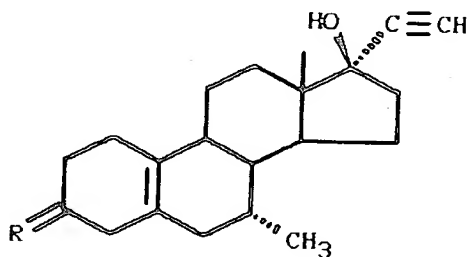
The invention relates to the use of pregnane derivatives for the manufacture of a medicament for the prevention or treatment of tumors.

In the endocrine therapy of breast cancer, patients may be treated with hormones, like progestogens (G.H. Bakker et al. in *Hormonal Manipulation of Cancer: Peptides, Growth Factors, and New (Anti) Steroidal Agents*, edited by Jan G.M. Klijn et al., Raven Press, New York, 1987, p. 39) and androgens (M.N. Teller et al., *Cancer Res.* 26, No.2, Pt.1, 245, 1966; S. Dauvois et al., *Ann.N.Y.Acad.Sc.* 595, 413, 1990). Cancer treatment with progestogens gives, however, undesirable side-effects, especially when applied in high dosages, such as abdominal distension and pain, nausea, headache, depression, and the like. When androgens are applied, also a number of unfavourable side-effects occurs, of which virilizing phenomena like hoarseness, hirsutism and alopecia are most frequently observed.

The use of other drugs not having the above-mentioned undesired side-effects would be highly favourable. However, it is known that such drugs are not permitted to have estrogen activity: drugs with estrogen activity cannot be used in patients having breast cancer due to the apparent estrogen sensitivity of mammary tumors (R.W. Brueggemeier et al., *Cancer Research* 48, 6808, 1988; Y.J. Abul-Hajj, *J. Steroid Biochem.*, 34, 439, 1989).

We now have found compounds which are suitable for preventing or treating cancer, in particular mammary tumors, with improved properties with respect to side-effects.

The invention relates to the use of pregnane derivatives of the following general formula:



in which R = H₂, (H,OH), (H,OAcy), or O, for the preparation of a medicament for the prevention or treatment of tumors. These compounds, in particular the derivatives in which R = (H,OH) or O, and especially the pregnane derivative in which R = O, (7 α ,17 α)-17-hydroxy-7-methyl-19-nor-17-pregn-5(10)-en-20-yn-3-one (compound I), have in rats a clearly established estrogen activity, apart from a very weak androgen activity; progestogenic activity could not be demonstrated in this species (J. de Visser et al., *Arzneim. Forsch.* 34, 1010, 1984). Although it can be anticipated that the estrogen activity of this compound would prevent its application in breast tumor therapy, it is surprisingly found that this compound has no negative estrogen-like, tumor-increasing effects on DMBA-induced mammary tumors in rats. Contrary to expectation, tumor growth was significantly decreased on treatment with the compound of the invention. This compound can, therefore, be used as a medicament in anti-tumor therapy without having unfavourable side-effects.

The term acyl means an acyl group derived from an organic carboxylic acid having 1-18 carbon atoms, such as formic acid, acetic acid, propionic acid, butyric acid, valeric acid, palmitic acid, phenylpropionic acid, maleic acid and citric acid. Preferred acyl groups have 1-6 carbon atoms, and most preferred is the acetyl group.

Compound I is a known compound, the synthesis of which is described e.g. in US Patent Application Publication No. 3,340,279. Preferably, the crystalline pure monoclinic (P2₁) form of (7 α ,17 α)-17-hydroxy-7-methyl-19-nor-17-pregn-5(10)-en-20-yn-3-one (tibolone, compound II) is used, because of its improved stability, bioavailability and shelf-life. The synthesis and use in a pharmaceutical preparation of this monoclinic derivative is disclosed in European Patent Application Publication No. 0,389,035.

The compound of the invention may be administered enterally or parenterally, and for humans in a daily dosage of 0.003-3.0 mg per kg body weight; preferably a daily dosage of 0.03-0.4 mg per kg body weight is administered. Mixed with pharmaceutically suitable auxiliaries, e.g. as described in the standard reference, Gennaro et al., *Remington's Pharmaceutical Sciences*, (18th ed., Mack Publishing Company, 1990, see especially Part 8: Pharmaceutical Preparations and Their Manufacture) the compound may be compressed into solid dosage units, such as pills, tablets, or be processed into capsules or suppositories. By means of pharmaceutically suitable liquids the compound can also be applied as an injection preparation in the form of a solution, suspension, emulsion, or as a spray, e.g. a nasal spray. For making

dosage units, e.g. tablets, the use of conventional additives such as fillers, colorants, polymeric binders and the like is contemplated. In general, any pharmaceutically acceptable additive which does not interfere with the function of the active compound can be used.

Suitable carriers with which the compositions can be administered include lactose, starch, cellulose derivatives and the like, or mixtures thereof, used in suitable amounts.

The invention is further illustrated by the following examples.

Example 1

A tablet having the following composition was prepared:

| | |
|--------------------|----------------------|
| compound I | 2.5 mg |
| starch | 10 mg |
| ascorbyl palmitate | 0.2 mg |
| magnesium stearate | 0.5 mg |
| lactose | to make up to 100 mg |

Base granules were prepared by mixing the lactose with a portion of the starch. The remainder of the starch was mixed to a slurry with water and added to the mixture. The whole was granulated and dried. These base granules were mixed with ascorbyl palmitate and compound I, sieved, finely mixed with magnesium stearate and then tabletted.

Example 2

A tablet having the following composition was prepared:

| | |
|--------------------|----------------------|
| compound II | 2.5 mg |
| starch | 10 mg |
| ascorbyl palmitate | 0.2 mg |
| magnesium stearate | 0.5 mg |
| lactose | to make up to 100 mg |

The preparation of the tablet was performed according to the procedure of Example 1.

Example 3

A tablet having the following composition was prepared:

| | |
|--|----------------------|
| (7 α ,17 α)-3,17-dihydroxy -7-methyl-19-nor-17-pregn-5(10)-en-20-yn | 2.5 mg |
| starch | 10 mg |
| ascorbyl palmitate | 0.2 mg |
| magnesium stearate | 0.5 mg |
| lactose | to make up to 100 mg |

The preparation of the tablet was performed according to the procedure of Example 1.

Example 4

A tablet having the same composition as in Example 1 was prepared by first mixing compound I with 10% of the lactose and the ascorbyl palmitate and then mixing this mixture with the lactose, starch and starch slurry. The mixture was dried, finely mixed with magnesium stearate and tabletted.

Example 5

A tablet having the same composition as in Example 2 was prepared by first mixing compound II with 10% of the lactose and the ascorbyl palmitate and then mixing this mixture with the lactose, starch and starch slurry. The mixture was dried, finely mixed with magnesium stearate and tableted.

Example 6

Four separate experiments were performed (i - iv) with groups of female rats (Sprague-Dawley; age 55-60 days). The number of rats per group was 8. Induction of mammary tumors was performed by two oral administrations of 1 ml of dimethylbenzanthracene (DMBA 10 mg/ml in olive oil) given with 1 week interval.

At an age of 105-115 days when all rats had palpable tumors, the rats were treated orally twice daily with vehicle (control groups) or with compound II (week 0). Because tumors were smaller of volume at the above mentioned age, treatment in experiment iv started when the animals were 115-122 days of age. Compound II was administered orally by gavage as a suspension in 0.5% gelatin and 5% mannitol (w/v) in water (volume: 1 ml/kg). The daily doses of compound II which were administered for 3 weeks, were 2 x 0.125, 2 x 0.25, 2 x 0.5 or 2 x 1 mg/kg/day. The rats of the control groups were treated orally with vehicle only (0.5% gelatin and 5% mannitol in water), twice daily for 3 weeks. Volume 1 ml/kg.

Before treatment, the rats were palpated weekly for the presence of tumors. After one and two weeks of treatment the tumors were weekly measured under light anesthesia using callipers. The total tumor load per rat represents the sum of the individual areas being the product of the perpendicular diameters. On the last day of treatment the animals were killed under deep anesthesia. At autopsy blood was collected and plasma was assayed for levels of LH, FSH, estradiol, progesterone, corticosterone, ACTH and prolactin. The tumors were measured as described above, dissected free from connective tissue and weighed.

| Results | | | | | | |
|--------------|------------------|---------------------------------|------|------|------|--------------|
| | Twice daily dose | Tumor burden (mm ²) | | | | Tumor weight |
| Experiment | (mg/kg) | wk 0 | wk 1 | wk 2 | wk 3 | (mg) |
| i. Control | -- | 288 | 625 | 1051 | 1743 | 21300 |
| II | 1.0 | 289 | 375 | 422 | 491 | 6130 |
| ii. Control | -- | 291 | 534 | 805 | 1070 | 11068 |
| II | 1.0 | 289 | 292 | 343 | 358 | 4800 |
| iii. Control | -- | 288 | 620 | 1060 | 1530 | 12700* |
| II | 0.125 | 299 | 620 | 1060 | 1370 | 9300 |
| II | 0.25 | 226 | 490 | 930 | 1190 | 11000* |
| II | 0.5 | 319 | 770 | 900 | 840 | 6500 |
| iv. Control | -- | 341 | 800 | 1440 | 2090 | 20700* |
| II | 0.25 | 474 | 760 | 990 | 1290 | 13400 |
| II | 0.5 | 474 | 610 | 890 | 1140 | 11000 |
| II | 1.0 | 323 | 570 | 870 | 1040 | 12000 |

* group of 7 rats

Conclusion: The pregnane derivative of the invention, administered twice daily gives a lower tumor load compared to the control group. A twice daily dose of 1 mg/kg inhibits the tumor growth in rats up to about 70%.

Example 7

An experiment was performed with 35 female rats (Sprague-Dawley; age 55-60 days). The rats were divided into 4 groups (number of rats per group: 8-9) according to a randomized block design (3 rats per cage). Induction of mammary tumors was performed by two oral administrations of 1 ml of dimethylbenzanthracene (DMBA 10 mg/ml in olive oil) given with 1 week interval. One group was ovariectomized (OVX-group) and used as reference group.

From the first day of DMBA-treatment, the rats were treated orally with vehicle (control groups and the OVX-group) or with compound II. Compound II was administered orally twice daily by gavage, as a suspension in 0.5% gelatin and 5% mannitol (w/v) in water (volume: 1 ml/kg). The daily doses of compound II which were administered for 10 weeks, were 2 x 0.25 or 2 x 1.0 mg/kg/day. The rats of the control and reference groups were treated orally with vehicle only (0.5% gelatin and 5% mannitol in water, volume: 1 ml/kg), twice daily for 10 weeks.

The rats were weekly palpated for the presence of tumors. From week 7 onwards the tumors were weekly measured under light ether anesthesia using callipers.

The total tumor load per rat represents the sum of the individual areas being the product of the two largest perpendicular diameters. On the last day of week 10, the animals were killed under deep anesthesia. The tumors were measured as described above, dissected free from the connective tissue and weighed.

| Results | | | | | | |
|-----------|-----------|---------------------------------|------|------|-------|-------------------|
| | Dose | Tumor burden (mm ²) | | | | Tumor weight (mg) |
| | mg/kg/day | wk 7 | wk 8 | wk 9 | wk 10 | wk 10 |
| Control | | 400 | 718 | 1183 | 1761 | 10761 |
| II | 2 x 0.25 | 10.8 | 14.7 | 125 | 196 | 1161 |
| II | 2 x 1.0 | 52.4 | 120 | 242 | 322 | 1875 |
| OVX-group | | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

Conclusion: The pregnane derivative of the invention, administered twice daily at a low dose of 0.25 mg/kg, decreases the development of tumors in rats up to about 90%.

Example 8

An experiment was performed with 35 female rats (Sprague-Dawley; age 55-60 days). The rats were divided into 4 groups (number of rats per group: 8-9) according to a randomized block design (3 rats per cage). Induction of mammary tumors was performed by two oral administrations of 1 ml of dimethylbenzanthracene (DMBA 10 mg/ml in olive oil) given with 1 week interval. 24 h after the second DMBA-treatment, the rats were treated orally with vehicle (control groups) or with the pregnane derivative of this invention (compound II). The derivative was administered orally twice daily by gavage as a suspension in 0.5% gelatin and 5% mannitol (w/v) in water (Volume: 1 ml/kg). The daily doses of compound II which were administered for 9 weeks, were 2 x 0.063 or 2 x 0.25 mg/kg/day. The rats of the control and reference groups were treated orally with vehicle only (0.5% gelatin and 5% mannitol in water, volume: 1 ml/kg), twice daily for 9 weeks.

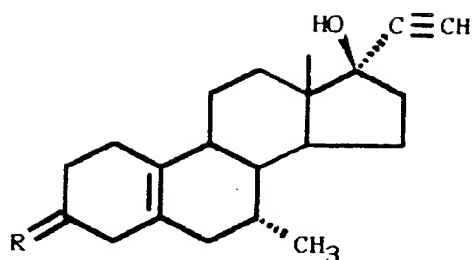
The experiment was further performed according to example 7.

| Results | | | | | | |
|---------|-----------|----------------------------------|------|------|-------|-------------------|
| | Dose | Tumour burden (mm ²) | | | | Tumor weight (mg) |
| | mg/kg/day | wk 7 | wk 8 | wk 9 | wk 10 | wk 10 |
| Control | | 314 | 683 | 1124 | 1544 | 6151 |
| II | 2 x 0.063 | 261 | 434 | 676 | 957 | 4179 |
| II | 2 x 0.25 | 107 | 360 | 591 | 825 | 3465 |

Conclusion: The pregnane derivative of the invention, administered twice daily at a low dose of 0.063 mg/kg, decreases the development of tumors in rats up to about 40%.

Claims

1. A use of a pregnane derivative of the general formula:



wherein R = H₂, (H,OH), (H,OAcyl), or O, for the manufacture of a medicament for the prevention or treatment of tumors.

2. The use according to claim 1 wherein the medicament is used for the prevention or treatment of mammary tumors.
3. The use according to claim 1 or 2 wherein R = (H,OH) ((7 α ,17 α)-3,17-dihydroxy-7-methyl-19-nor-17-pregn-5(10)-en-20-yn).
4. The use according to claim 1 or 2 wherein R = O ((7 α ,17 α)-17-hydroxy-7-methyl-19-nor-17-pregn-5(10)-en-20-yn-3-one (compound I)).
5. The use according to claim 4 wherein the pregnane derivative (7 α ,17 α)-17-hydroxy-7-methyl-19-nor-17-pregn-5(10)-en-20-yn-3-one has the monoclinic P2₁ form (compound II).
6. Method of treatment of tumors, comprising the administration of an effective amount of (7 α ,17 α)-17-hydroxy-7-methyl-19-nor-17-pregn-5(10)-en-20-yn-3-one.
7. Method of treatment of tumors, comprising the administration of an effective amount of (7 α ,17 α)-17-hydroxy-7-methyl-19-nor-17-pregn-5(10)-en-20-yn-3-one having the monoclinic P2₁ form.
8. Method of treatment of tumors, comprising the administration of an effective amount of (7 α ,17 α)-3,17-dihydroxy-7-methyl-19-nor-17-pregn-5(10)-en-20-yn.



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PARTIAL EUROPEAN SEARCH REPORT

Application Number

which under Rule 45 of the European Patent Convention EP 94 20 0523
shall be considered, for the purposes of subsequent
proceedings, as the European search report

| DOCUMENTS CONSIDERED TO BE RELEVANT | | | |
|--|--|----------------------------------|---|
| Category | Citation of document with indication, where appropriate, of relevant passages | Relevant to claim | CLASSIFICATION OF THE APPLICATION (Int.Cl.5) |
| A | EP-A-0 159 739 (AKZO N.V.) 30 October 1985 * the whole document * * especially page 7, table, first, third and fourth compounds * --- | 1-8 | A61K31/565 |
| A | EP-A-0 389 035 (AKZO N.V.) 26 September 1990 * the whole document * * especially page 2, line 20 and claim 8 * --- | 1-8 | |
| A | JOURNAL OF DRUG DEVELOPMENT vol. 4, no. 4, 1992 pages 235 - 244 SMITH, R. ET AL 'HORMONE REPLACEMENT THERAPY A REVIEW' * page 240, column 2, line 15 - page 242, column 2, line 3 * --- -/-- | 1-8 | |
| | | | TECHNICAL FIELDS SEARCHED (Int.Cl.5) |
| | | | A61K |
| INCOMPLETE SEARCH | | | |
| <p>The Search Division considers that the present European patent application does not comply with the provisions of the European Patent Convention to such an extent that it is not possible to carry out a meaningful search into the state of the art on the basis of some of the claims</p> <p>Claims searched completely : Claims searched incompletely : Claims not searched : Reason for the limitation of the search:</p> <p>see sheet C</p> | | | |
| Place of search | | Date of completion of the search | Examiner |
| THE HAGUE | | 6 June 1994 | Mair, J |
| CATEGORY OF CITED DOCUMENTS | | | |
| X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document | | | |
| T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document | | | |

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PARTIAL EUROPEAN SEARCH REPORT

Application Number
EP 94 20 0523

| DOCUMENTS CONSIDERED TO BE RELEVANT | | | CLASSIFICATION OF THE APPLICATION (Int.Cl.5) |
|-------------------------------------|---|-------------------|--|
| Category | Citation of document with indication, where appropriate, of relevant passages | Relevant to claim | |
| A | THE JOURNAL OF STEROID BIOCHEMISTRY vol. 35, no. 5 , April 1990 pages 535 - 541 MARKIEWICZ, L. ET AL 'IN VITRO EVALUATION OF ESTROGENIC, ESTROGEN ANTAGONISTIC AND PROGESTAGENIC EFFECTS OF A STEROIDAL DRUG (ORG OD-14) AND ITS METABOLITES ON HUMAN ENDOMETRIUM' * the whole document * | 1-8 | |
| A | BRITISH MEDICAL BULLETIN vol. 48, no. 2 , April 1992 pages 401 - 425 ELLERINGTON, M.C. ET AL 'HRT: DEVELOPMENTS IN THERAPY' * the whole document * * especially page 421, line 2-7 * | 1-8 | |
| | | | TECHNICAL FIELDS SEARCHED (Int.Cl.5) |
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EPO FORM 1503 01/92 (POMC/O)



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-C-

Remark: Although claims 6-8
are directed to a method of
treatment of the human/animal
body (Art. 52(4) EPC) the search
has been carried out and based on
the alleged effects of the
compound/composition

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